

THESIS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

Evaluating Swedish seaweeds for biorefinery

Species to use and ways to improve composition

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Cover: *Ulva fenestrata* in adaptation by the author. Photo provided by Gunnar Cervin

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“The greatest glory in
living lies not in never
falling, but in rising every
time we fall.”

-Nelson Mandela

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ABSTRACT

A sustainable future demands a transition from oil to biomass to produce fuels, chemicals, commodities, and energy. However, to generate the predicted amount of bioenergy required by 2050, all the biomass harvested today should be utilized. One type of biomass that has received relatively little attention is seaweeds. Seaweeds have many advantages over land-based biomass in that they do not require arable land or fertilizer, and their cultivation does not generally compete with food production. Only 20 out of 10 000 species are cultivated and the content of many of them remains poorly characterized. Understanding how their chemical composition is affected by abiotic factors will help determine which biomass component is most valuable and should be maximised.

This thesis aims to enable future research and development of a seaweed industry in Sweden. A screening of 22 species of seaweeds concluded that the most relevant species for biorefinery applications in Europe (*Saccharina latissima*, and *Laminaria digitata*) were also among the most interesting for Sweden. In addition, a few poorly investigated species were found to have interesting properties, such as high mannitol content (*Halidrys siliquosa*) as well as high sugar and low ash content (*Chondrus crispus* and *Dilsea carnosa*).

Composition of *Ulva intestinalis*, which grows all around Sweden, was studied in natural populations on the west coast and southern half of the east coast. Significant effects were found on carbohydrate, fatty acid and ash content between the sites. Rhamnose and iduronic acid were higher on the east coast, but not significantly. However, an elevated sulphate content motivates further investigation of the effect of salinity (the major differential environmental factor) on the potential high-value component ulvan in *Ulva intestinalis*. There were large variations in yields and composition of the oil, aqueous and solid phases in hydrothermal liquefaction processing within the different sites. Generally, the yields of bio-oil were low, and the quality of the oil was poor due to high contents of S, N and Fe. Considering the low quality of the oil, hydrothermal liquefaction should probably be utilized to treat side streams from a biorefinery after extraction of more high-value compounds.

Finally, abiotic factors and their effect on the growth and composition of *Ulva fenestrata* were studied. To overcome the low profitability projection of many biorefinery concepts, the data in this thesis could help maximize the value of algal biomass and launch a successful seaweed biorefinery industry. It was found that the content of the valuable monosaccharide rhamnose and the sugar acid iduronic acid could be increased by 26 and 70% respectively at elevated temperature and irradiance. This indicated an increase in ulvan content.

Keywords: Seaweed, macroalgae, carbohydrates, proteins, hydrothermal liquefaction, chemical composition, ulvan, variation, salinity, *Ulva* spp.

Preface

This doctoral thesis partly fulfils the requirements for a PhD degree at the Department of Biology and Biological Engineering, Chalmers University of Technology, Sweden. The work was performed between 2015 and 2020 and was funded by three different projects; Formas project number 2013-92 Seafarm, Swedish Foundation for Strategic Research (SSF) project number RBP14-0045 Sweaweed and the project “Alternativa biomassor som råvara för bioraffinaderikoncept” in the cooperation program “Preem and Chalmers towards a sustainable refinery” between Preem and Area of Advance-Energy at Chalmers. Seafarm and Sweaweed are both projects with multiple Swedish universities focused on different parts of the value chain from seaweed cultivation to a range of applications. The Preem project, with translated title “Alternative biomasses as raw material for biorefinery concepts”, was a collaborative project between the division of Industrial Biotechnology and division of Energy Technology where compositional analysis was combined with energy systems analysis and modelling. The collaboration with Prof. Chris Chuck and Dr Sofia Raikova at Bath University (UK) was set up within the Preem project to bridge the gap between composition and modelling with actual experiments.

The majority of the research presented in this thesis was performed at the division of Industrial Biotechnology at Chalmers University of Technology under the supervision of Associate Professor Dr Eva Albers. Supervision was also provided by Dr Göran Nylund at the Department of Marine Sciences - Tjärnö, Göteborg University and Professor Dr Ingrid Undeland at the division of Food and Nutrition Science, Chalmers University of Technology. There was also close collaboration with Associate Professor Dr Gunilla Toth and Dr Sophie Steinhagen at the Department of Marine Sciences - Tjärnö, Göteborg University.

Joakim Olsson
February 2020

List of publications

This thesis is based on the following papers, which are referred to in the text by their Roman numerals.

- I **Joakim Olsson**, Gunilla B. Toth and Eva Albers Biochemical composition of red, green and brown seaweeds common at the Swedish west coast. Manuscript, submitted Journal of Applied Phycology
- II **Joakim Olsson**, Sofia Raikova, Joshua J. Mayers, Christopher J. Chuck, Göran M. Nylund and Eva Albers Environmental effects on potentially valuable components of *Ulva intestinalis* along the Swedish coast. Manuscript
- III Sofia Raikova, **Joakim Olsson**, Joshua J. Mayers, Göran M. Nylund, Eva Albers, and Christopher J. Chuck. (2020) Effect of geographical location on the variation in products formed from the hydrothermal liquefaction of *Ulva intestinalis* Energy Fuels 34: 368-378 doi:10.1021/acs.energyfuels.8b02374
- IV **Joakim Olsson**, Gunilla B. Toth, Annelous Oerbekke, Suzana Cvijetinovic, Hanna Harrysson, Niklas Wahlström, Sophie Steinhagen, Alexandra Kinnby, Joel White, Ulrica Edlund, Ingrid Undeland, Henrik Pavia and Eva Albers Cultivation conditions affects the monosaccharide composition in *Ulva fenestrata*. Manuscript, submitted Journal of Applied Phycology

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Submitted manuscript not included in the thesis:

Gunilla B. Toth, Hanna Harrysson, Niklas Wahlström, **Joakim Olsson**, Annelous Oerbekke, Sophie Steinhagen, Alexandra Kinnby, Joel White, Ingrid Undeland, Ulrica Edlund, Eva Albers and Henrik Pavia Effects of irradiance, temperature, nutrients and pCO₂ on the growth and biochemical composition of *Ulva fenestrata*. Manuscript, submitted Journal of Applied Phycology

Author's contributions

Paper I: First author. Planned and performed the experimental work. Wrote the manuscript.

Paper II: First author. Conceived and planned the study. Performed much of the experimental work, analysed most of the data and wrote the manuscript.

Paper III: Second author. Conceived the idea, and was responsible for setting up the collaboration that finalized the idea, and planned the study. Proofread the manuscript.

Paper IV: First author. Planned the carbohydrate analysis and performed most of the experimental work. Partook in some data analysis and wrote the manuscript.

Abbreviations

AEC	anion-exchange chromatography
ÅHS	Åhus
dw	dry weight
FTIR	Fourier-transform infrared spectroscopy
GAG	glucosaminoglycans
Gal	galactose
GBG	Göteborg
Glc	glucose
GlcA	glucuronic acid
GulA	guluronic acid
HBG	Helsingborg
HPAEC-	high-performance anion-exchange chromatography with pulsed amperometric
PAD	detection
HPLC	high-performance liquid chromatography
HTL	hydrothermal liquefaction
IdoA	iduronic acid
KKR	Karlskrona
ManA	mannuronic acid
NMR	nuclear magnetic resonance
PC	principal component
PCA	principal component analysis
PUFA	polyunsaturated fatty acid
Rha	rhamnose
RI	refractive index
STH	Stockholm
TBG	Trelleborg
VSV	Västervik
Xyl	xylose

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1. Introduction

Just like other organisms, humans shape the environment in which they live and, sometimes, that can lead to unwittingly damaging our habitat. An early example was the Mesopotamian civilization, which was built on the fertile lands around the Euphrates and Tigris rivers, and thrived thanks to an extensive system of water channels and dams. However, over the centuries, the water used for irrigation deposited salt in the fertile farmlands until they were no longer suitable for cultivation and cities were abandoned (McIntosh 2005). Similarly, in spite of its improvements to the quality of life, the industrial revolution changed our environment in a deteriorating direction. Unlike the people in Mesopotamia, we have been aware of a potential danger to our civilization since 1859, when John Tyndall discovered the greenhouse effect and theorized about its possible impact on our planet (Hulme 2009). However, unlike the people of ancient Mesopotamia we cannot simply move, and must instead find a way to intelligently solve our crisis. One solution is to transition towards a more sustainable bioeconomy.

Seaweeds have received sustained interest in recent years for their potential within a future bioeconomy. Like terrestrial plants, seaweeds use photosynthesis to convert CO₂ and nutrients into oxygen and biomass and, therefore, have the potential to participate in a circular system with little negative effect on the environment. Unlike crops, which have been cultivated for thousands of years, the first record of seaweed farming dates to 1670 in Tokyo Bay. Hence, it comes as no surprise that there are only a few species, for which cultivation methods exist. Moreover, only a fraction of the known 10 000 species of seaweeds have been characterised (Guiry and Guiry 2018). Owing to their diverse evolutionary history and lack of the universal structure typical of lignocellulosic biomass, seaweeds represent an almost untapped source of novel raw materials, bioactive substances, and food or feed ingredients.

An important concept of the future bioeconomy is the biorefinery, whereby biomass constituents are extracted and separated into products or raw materials. Several products of interest for the biorefinery concept can be found in seaweeds and a large research effort is underway to improve the extraction of sugars, proteins, lipids, and other components. While, there are several successful businesses extracting components from seaweeds, extraction of bulk products such as ethanol has not been found profitable (Konda et al. 2015). Knowing that the composition of seaweeds varies with environmental conditions, it could be possible

to make a better economic case for seaweed exploitation by maximizing the components with higher added value in response to abiotic stimuli.

Bioenergy is an important element of a future circular economy. In an IPCC report from 2012, 150–300 EJ y⁻¹ was suggested for 2050, which is more than the total biomass production for food, fodder, and fibre (219 EJ y⁻¹) in the year 2000 (Chum et al. 2011). Importantly, that report excluded aquatic biomasses due to existing uncertainty regarding their availability and yield. Hence, seaweeds could potentially close that gap and reduce the burden on land-based ecosystems. Admittedly, seaweeds have been found to be unsuitable for some common methods of energy extraction, such as combustion and gasification (Ross et al. 2008). This is primarily due to their high percentage of water, which has to be removed prior to these processes, but there are also potential issues related to their high ash and low energy contents. These drawbacks could be overcome by methods such as hydrothermal liquefaction (HTL), which are less sensitive to ash and can be carried out on wet biomass.

Seaweeds are not traditionally used in Swedish cuisine and are mostly something people find repelling when it gets entangled in their legs while swimming. However, Sweden has a long coastline, with 500 km² available for cultivation in the northern part of the west coast alone (Thomas et al. 2019). Before that can happen, though, it needs to be determined what species should be utilised and at what locations they should be grown to obtain the most valuable biomass. Within this thesis work I have made an effort to, in part, answer these questions.

1.1 Aims

The overarching aim of my thesis work can be summed up by the following question: how to enable seaweeds to be part of a future bioeconomy? To achieve this goal, some key knowledge gaps were identified: 1) we know the composition of too few species to fully evaluate seaweeds potential, 2) we do not know enough about how composition affects processing, and 3) we do not know how abiotic cultivation factors impact valuable content in seaweeds.

Paper I

This paper focuses on evaluating the composition of some seaweeds commonly found along the Swedish west coast. The aim was to evaluate their potential usefulness for biorefinery by screening the most abundant seaweed compounds (ash, carbohydrates, and proteins). While the results may be insufficient to draw conclusions about the potential of specific species, part of the goal was to identify promising species to focus on in further studies, such as those in Papers II-III.

Paper II

From Paper I, several interesting species were identified, including some that had been already extensively characterised. As the goal of the project behind this paper was to fill existing knowledge gaps, the study focused on *Ulva intestinalis*. The specific aim was to evaluate the composition of this species around the Swedish coast and thus gain insight into which environmental factors influenced the high-value products in this seaweed. This could help identify locations for cultivation trials or starting conditions for tank cultivation.

Paper III

Using the same samples as Paper II, this study aimed to evaluate whether compositional changes had any effect on processing of the seaweeds. As this was a collaborative project with partners working on energy systems analysis, HTL was chosen and external partners at Bath University had the equipment to perform the necessary experiments.

Paper IV

From Paper I, *Ulva fenestrata* (previously *Ulva lactuca*) was identified as another interesting species and became the focus of a large collaborative project. The aim of the study was to evaluate the effect of abiotic factors on monosaccharide composition and ulvan structure. In particular, the study assessed factors relevant to the local area around Tjärnö Marine Laboratory (TML, 58°52'36.4"N11°6'42.84"E), which is why salinity was not included despite being identified as an important factor in Paper II.

2. Bioeconomy and biorefinery

The European Commission has set a bioeconomy policy that defines bioeconomy as “a resource-efficient and sustainable economy. The goal is a more innovative and low-emissions economy, reconciling demands for sustainable agriculture and fisheries, food security, and the sustainable use of renewable biological resources for industrial purposes, while ensuring biodiversity and environmental protection.” To ensure growth of the bioeconomy, the technologies and processes to convert bio-based raw materials into value-added products need to be developed and the required raw materials have to be available.

2.1 The biorefinery

The definition of biorefinery remains a matter of perspective but, as a general rule, it applies to any industrial process that upgrades biomass. Technically, this would make even a steel mill, where coal is replaced with biomass, a biorefinery as the biomass, albeit together with iron, is upgraded to steel. Therefore, I would rather define the biorefinery as a sustainable process, in which the main raw material is biomass. In Figure 1, a schematic of the envisioned value-chain of a biorefinery utilising seaweeds in the Seafarm project.

Fermentation

For biorefinery purposes, fermentation is almost exclusively referred to as the conversion of carbohydrates by microorganisms, such as *Saccharomyces cerevisiae*, *Lactobacillus* spp., *Clostridium acetobutylicum*, and *Escherichia coli*, into products. Typical products obtained from carbohydrates using seaweeds include ethanol, butanol, and succinate (Adams et al. 2009; Huesemann et al. 2012; Marinho et al. 2016), but generally any small organic molecule or protein that can be produced by an organism is a potential product for fermentation of carbohydrates. However, the carbohydrates have to be in a fermentable form, which in most cases means as monosaccharides and hence polysaccharides have to be broken down through pre-processing. The production organism must also have the genetic tools for the degradation and uptake of monosaccharides, as well as the ability to withstand inhibitors while staying productive.

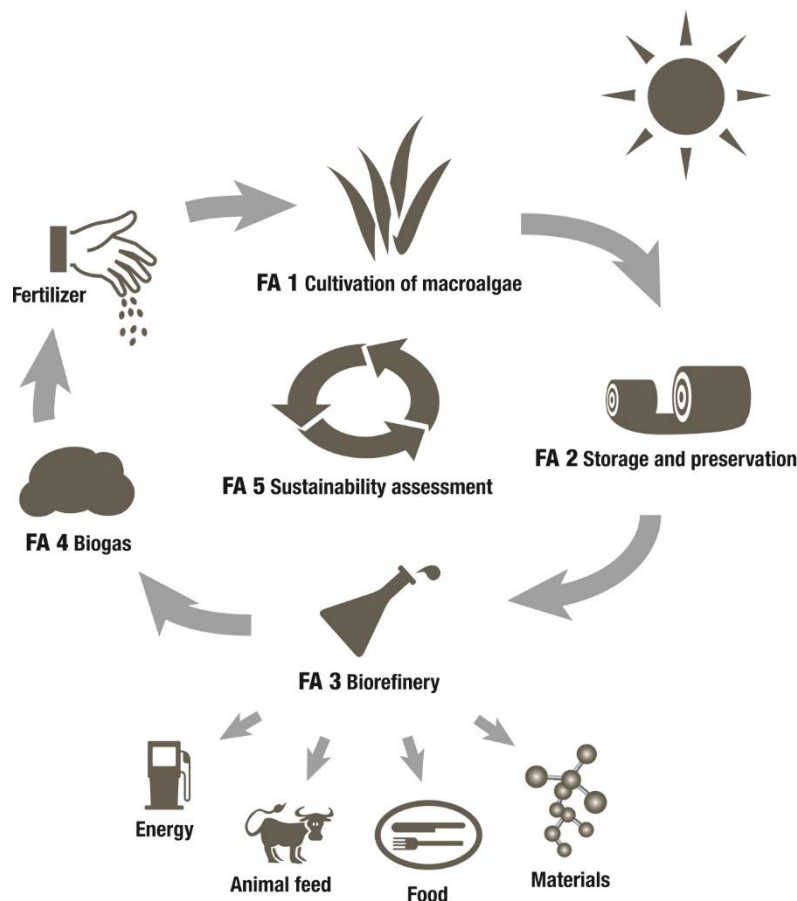


Figure 1. Graphic overview of the seaweed value-chain and focus areas of the Swedish research project Seafarm (Gröndahl et al. 2013).

Bioenergy

There are many different processes for energy production and, given the elevated water content of seaweeds, they can be divided in dry and wet methods. Dry methods include direct combustion, pyrolysis, and gasification. These methods are all technologically ready but are not very suitable for seaweeds, largely due to the high energy cost of drying seaweeds, as well as the ash components that can cause fouling of boilers, reduce bio-oil yield and quality (Milledge et al. 2014).

Wet methods for energy extraction from seaweeds include the production of bioethanol and biobutanol, as mentioned under the previous heading, as well as HTL and anaerobic digestion. HTL is similar to pyrolysis, but is performed at higher pressure of 4–22 to 0.1–0.5 MPa and lower temperature of 250–374 to 450–525 °C, and the raw material is wet (Demirbaş 2001; Gollakota et al. 2018). The method decomposes the biomass into bio-oil as well as aqueous, gaseous, and solid (biochar) phases. High-quality bio-oil low in contaminating nitrogen, sulphur, and minerals can be fed into an oil refinery and upgraded as done with crude oil. The aqueous phase can also have value as nitrogen and phosphorus sometimes accumulate in it, allowing nutrients to be recycled.

Fractionation

This biorefinery concept is based on extracting the different fractions in the targeted biomass into useful products or ingredients. Sometimes called a cascading biorefinery, it represents the approach that can extract the most value from biomass. However, biorefineries based on

fractioning compounds sometimes rely on a complicated scheme, whereby chemicals used for extraction could limit the potential environmental gain from using biomass. There are, nevertheless, examples where this has been considered (Stern et al. 2017). One should keep in mind that the fractions do not necessarily have to be pure, because combined fractions such as those consisting of lipids and proteins could have value as a food or feed ingredient. The potential inclusion of anaerobic digestion or HTL as final steps in a biorefinery concept would allow the utilisation of side streams, further promoting a circular economy.

2.2 Biomass needs and available raw materials

At present, it is not easy to predict the amount of biomass required in the future and there are large uncertainties when it comes to future technological development as well as increases in crop yields. Competition for land could potentially lead to deforestation, food shortages, and general unsustainable practices (Searle and Malins 2015). However, seaweeds are often not included in predictions of future biomass availability and need as they are deemed too technologically immature (Chum et al. 2011). To lessen the burden on specific system, and safeguard the availability of food, the range of usable raw materials should be as wide as possible include also seaweeds. Seaweeds and their composition are discussed in section 3.1

Available materials

Several different raw materials are available for biorefinery purposes, but large hopes have been put in various lignocellulosic biomasses. The amount of carbohydrates and ash of plant-based biomasses commonly used in biorefinery are listed in Table 1; the remaining content corresponds to lignin and other extractives, such as fats and proteins. Lignocellulosic raw materials are technologically ready for utilisation in biorefineries and there are several commercial plants in operation.

Table 1. Composition of commonly available land-based biomasses for biorefinery

	Carbohydrates (%)	Ash %	Ref
Bagasse	66.7	4.8	(Kim and Day 2011)
Corn Stover	67.3	4.3	(Troger et al. 2013)
Rape stalks	54.0	7.8	(Troger et al. 2013)
Sunflower stalks	51.0	14.4	(Troger et al. 2013)
Wheat straw	61.1	6.8	(Troger et al. 2013)
Softwood	60.8	0.5	(Troger et al. 2013)

Economy of biorefineries

The current success, or lack thereof, of biorefineries depends largely on the definition of what a biorefinery is, as discussed earlier. Disregarding already established businesses such as first-generation ethanol plants and paper mills and focusing on emerging technologies, the lignocellulosic bioethanol plants are a prime competitor of seaweed biorefineries. In recent years several such biorefineries in commercial scale have been opened and, as is the case for refineries by Dow-DuPont (Nevada, USA) and Beta Renewable (Cresentino, Italy), closed. The production costs have been too high and the gasoline prices too low (Chandel et al. 2018). These issues are going to be the same for seaweed biorefineries if the target is bulk products, such as ethanol. For now biomass is too expensive in developed countries and technological development is needed for efficient and less labour intensive harvesting of

seaweeds (Palatnik and Zilberman 2017). There is, however, some agreement among researchers that high-value products from algae will be vital for the economic viability of algae biorefineries and allow co-production of biofuels. This is already showcased by a plant outside of Copenhagen where the carrageenan producer CP Kelco's residues are transported to a nearby biogas facility where it is converted, together with other industrial residuals, into biogas and fertiliser (Solrød Biogas 2016).

3. Seaweeds and their potential in Sweden

Potential is defined as the ability to succeed and seaweeds seem to have the potential to succeed as an industry in Sweden. There are about 500 km² of suitable space along the Swedish Skagerrak coast alone for the cultivation of *Saccharina latissima* and the public perception towards increased aquaculture in this region is positive (Thomas et al. 2018; Thomas et al. 2019). However, consistency in quality and composition have been identified as key factors for the future seaweed industry to become successful (Hafting et al. 2015), and seaweeds are known to vary in composition and growth characteristics depending on several abiotic factors. To achieve consistent quality and composition we need to know the impacts of abiotic factors in detail. Some environmental factors vary strongly in Swedish waters, especially when the entire coast is factored in. There are 375 species of macroalgae to be found in Swedish waters and only a few of them have been explored (Naturvårdsverket 2009). To identify the most suitable species for biorefinery purposes, it is necessary to look for more candidates, as well as to explore compositional diversity across species and geographic locations.

3.1 Seaweeds

There are approximately 10 000 species of seaweeds, or marine macroalgae, that belong to the three groups: Rhodophyta (red), Phaeophyta (brown), and Chlorophyta (green) (Guiry and Guiry 2018). Having adapted almost exclusively to a marine environment, seaweeds display an extremely diverse life cycle and composition. They are, in fact, much more diverse than land-based plants, which diverged from algae 500 million years ago. At that point seaweeds had already been evolving and diverging for over a billion years (Pires and Dolan 2012). Whereas land-based plants are often called lignocellulosic biomasses in technical contexts because of shared structural elements, such as cellulose, hemicellulose, and lignin, this is not the case for seaweeds, whose components can vary greatly.

Seaweed composition

While all life is made of carbohydrates, proteins, lipids, and ash, their relative amounts vary greatly. A meta-study by Fiset et al. (2019) revealed that the median seaweed was composed of 9.98% proteins, 2.7% lipids, 48.5% carbohydrates, and 31.8% ash as percentages of dry weight. These data allow some general conclusions regarding seaweed composition to be drawn. Ash content is very high in comparison to other biomasses, which is, in large, an effect of seawater salinity. In contrast, lipid and protein contents are generally low, while carbohydrates are usually the main fraction on a dry weight basis. Lastly, a general feature of seaweed biomass is the high moisture content, estimated at 61–94% (Holdt and Kraan 2011).

Seaweed polysaccharides

Polysaccharides play different roles in a cell and can usually be classified as storage or structural carbohydrates. In seaweeds, the specific sugars vary substantially between green, red, and brown macroalgae, making them a large prospecting pool of potentially bioactive carbohydrates. Some of the more common ones are listed in Table 2. In addition, some monosaccharides from seaweeds, such as rhamnose and iduronic acid, are not commonly found elsewhere in nature, and could be a source of high-value products from seaweeds.

Table 2. Examples of common structural and storage carbohydrates in seaweeds. Abbreviations used: Guluronic Acid (GulA), Mannuronic Acid (ManA), Galactose (Gal), Glucose (Glc), Mannitol (ManOH), Rhamnose (Rha), Xylose (Xyl), Iduronic Acid (IdoA), Glucuronic Acid (GlcA),

Polysaccharide	Monosaccharides	Linkages	Approx. dw (%)	Ref
<u>Structural carbs</u>				
Alginate	GulA, ManA	α -(1-4), β -(1-4)	10-47	(Holdt and Kraan 2011)
Carrageenan	Gal	β -(1-4), α -(1-3)	47-88	(Holdt and Kraan 2011)
Cellulose	Glc	β -(1-4)	2-10	(Holdt and Kraan 2011)
Glucuronan	GlcA	β -(1-4)	2.5	(Redouan et al. 2009)
Ulvan	Rha, Glc, Xyl, GlcA, IdoA	α -(1-4), β -(1-4)	9-36	(Kidgell et al. 2019)
<u>Storage carbs</u>				
Floridean starch	Glc	α -(1-4), α -(1-6)	25-42	(Holdt and Kraan 2011)
Laminarin	Glc, ManOH	β -(1-3), β -(1-6)	1-25	(Schiener et al. 2015)
Starch	Glc	α -(1-4), α -(1-6)	2-21	(Prabhu et al. 2019)

An example of a seaweed cell wall is illustrated in Figure 2. On the one hand, such structure provides an advantage over lignocellulosic biomasses during fermentation because seaweeds generally lack the recalcitrant lignin that forms inhibitory compounds during pretreatment. On the other hand, it also contains less cellulose and the diverse monosaccharide profile puts high demands on the production organisms' range of substrates. Additionally, glucose, which is the easiest sugar for fermentation, is found in some of the storage carbohydrates that fluctuate seasonally, as described by Vilg et al. (2015). Another issue is that the pretreatment step required to release monosaccharides is not sufficiently developed for seaweeds and this has led to insufficient levels of sugar to make ethanol production profitable (Yanagisawa et al. 2013).

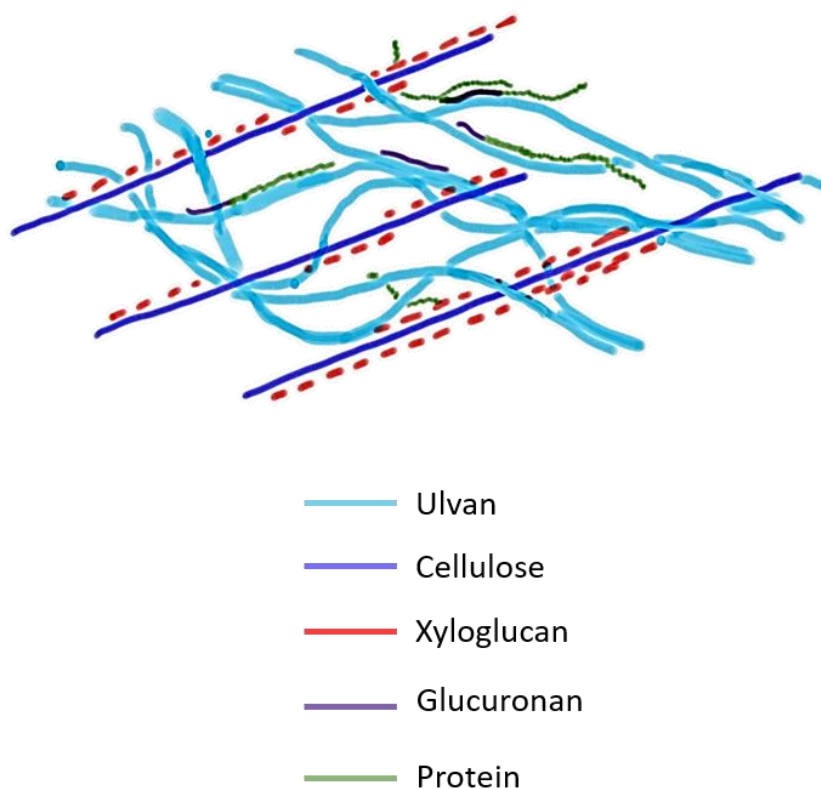


Figure 2. Schematic representation of the cell wall of *Ulva* spp. redrawn from Lahaye and Robic (2007) by Venkat Rao Konasani

Availability and sustainability

Seaweeds are obtained in two different ways, either by harvesting wild algae or through aquaculture cultivation. Wild algae have been harvested for many years by small businesses along the coast of Ireland without negative effects on long-term productivity. In contrast, it can take over 5 years for the kelp forest along the Norwegian coast to recover from harvesting (Steen et al. 2016) and these important habitats are already being impacted by climate change (Smale et al. 2013). There is also the possibility to use beach cast seaweeds, but during ensilage experiments by the author there were big losses of laminarin and proteins in untreated wet seaweeds (Olsson et al. unpublished data). Cultivation, in comparison, generates fresh biomass while potentially providing habitats as well as other ecosystem services (Hasselstrom et al. 2018). Though, drawbacks may come from competition with other uses of the sea, such as recreation, as well as the costs for cultivation infrastructure. Another positive effect of seaweeds is that they do not need fertilisers and are more productive than rain forests (Leigh et al. 1987). Hence, seaweeds combat both ocean acidification and eutrophication by sequestering nutrients from their surroundings.

In 2017, the global harvest of aquacultured seaweeds was 31.7 million tonnes, whereas only 1.1 million tonnes were harvested from the wild (FAO 2017). The majority are cultured in China and all top producers of cultured seaweeds are Asian. Given the growing demand for seaweeds and the availability of water resources, there is potential for the volumes to grow. Limitations other than space, however, could hamper growth as discussed in section 2.

Current uses of seaweeds

When considering seaweeds as a potential future resource, it is easy to forget that there already is a thriving industry. Seaweeds are part of the traditional cuisine in many countries, particularly in East Asia. Direct use in food is the major utilisation of seaweeds today. Carrageenan, agar, and alginate are extracted for their properties as hydrocolloids for use as texture agents, stabilisers, and paper coatings. In 2009, the total sales volume of seaweed hydrocolloids was 86 100 tonnes (Bixler and Porse 2011). Recently, seaweeds have also received attention by the cosmetics industry for their bioactive compounds and they have entered the formulation of several products.

Novel utilisation of seaweeds

In my opinion, recently explored seaweed products fall in two categories: bulk products such as ethanol, and high-value products such as bioactive polysaccharides. While a market for bulk products already exists, it could be difficult to achieve profitability, see end of section 2. In contrast, high-value products have been shown to possess similar activities as existing compounds that fetch a high price, but do not have a market yet. Moreover, the price of high-value products can drop quickly depending on the produced volume, as reported by Konda et al. (2015), who studied co-production of ethanol and alginate. Accordingly, a single commercial-scale ethanol plant would saturate the market for alginate and question the profitability of the process. To avoid this fate, a biorefinery approach with several products, including various high-value products and one bulk product such as biogas, bio-oil or ethanol, should be attempted. Crucially, efforts should be focused on more novel applications than alginate, and possibly on products that can replace environmentally troublesome items.

One suitable candidate relevant to the work in this thesis is ulvan. Ulvan and the oligosaccharides derived from it have been found to possess antioxidant, anticoagulative, immunostimulative, immunomodulative, cancer chemopreventative, and cytotoxic properties (Abd-Ellatef et al. 2017; Castro et al. 2006; Hussein et al. 2015; Kaeffer et al. 1999; Kim et al. 2011a; Leiro et al. 2007; Mao et al. 2006; Qi et al. 2005a; Qi et al. 2005b; Tabarsa et al. 2012). Several studies such as Kim et al. (2011b), have demonstrated the use of starch present in *Ulva* spp. for ethanol production, while ulvan, glucuronan, and cellulose could generate a range of additional products (Redouan et al. 2009; Wahlström et al. 2020). Seaweeds generally contain essential amino acids as well as polyunsaturated fatty acids (Fleurence et al. 2018; Holdt and Kraan 2011), which are low in content but could be extracted to add value to a process. Whereas *Ulva* is not the only genus suitable for a fractionation concept, whereby all its components are extracted and utilised, it is the most relevant to this thesis. But, species such as *S. latissima* have also been explored by many (van Hal et al. 2014).

3.2 Swedish species for biorefinery

Not all 375 species available in Swedish waters will be suitable for biorefining and there are several considerations to be made regarding the choice of species. One is that the species needs to be either sustainably harvested from natural stocks or be culturable. Both would require permits and the Seafarm project found that, to obtain such a permit, any seaweed cultivation had to be based on specimens from the local gene pool. Therefore, **Paper I** focused on species that were commonly available in the area without making further considerations regarding the potential of obtaining sufficient volumes of biomass for a future biorefinery concept. Nevertheless, based on their commonality, it was assumed that the included species could potentially supply a biorefinery.

Given the special environment discussed in section 4 and very limited studies on seaweed composition in Swedish waters, the composition of algae was screened to identify potentially suitable species. As many species as was practically possible were included. Due to the large number of species (22, see Table 1, **Paper I**), common bulk methods for the measurement of sugars (reducing sugar assay) and protein (N-factor conversion) were chosen. This sacrificed resolution compared to alternative methods discussed in section 6, but allowed the inclusion of species not previously studied without the need for extensive method development. Lipids were completely omitted in this study because of potential methodological issues with new species and their low content in most seaweeds. Species such as *Saccharina latissima* and *Laminaria digitata* had already been characterised as having the same high carbohydrate content, which promoted their study in many places around Europe (Manns et al. 2017; Schiener et al. 2015; Vilg et al. 2015). Therefore, these commonly researched species were included as a reference to compare species with unknown composition.

The data from **Paper I** are illustrated in Figure 3, which clearly shows that the two dominating fractions were ash and carbohydrates, in accordance with published reports. However, the mean carbohydrate content was well below the median 48.5% reported in the literature (Fiset et al. 2019). Among the species studied, only *Saccharina latissima*, *Laminaria digitata*, and *Chondrus crispus* were above this median, while *Dilsea carnosa* was very close. This might point to a generally lower carbohydrate content for algal species in Swedish waters or, more likely, to the general lack of studies on carbohydrate content in seaweeds. The meta-analysis by Fiset et al. (2019) included only 58 studies on carbohydrate levels. Moreover, it considered studies where carbohydrate content was determined by difference, *i.e.*, protein, lipid, and ash content were determined and carbohydrates were assumed to make up the rest. Such studies likely overestimated carbohydrate content, leaving plenty of room for its correct determination by future investigations. It is, however, clear that some seaweeds do have high enough carbohydrate contents to compete with lignocellulosic biomasses, see Table 1, but their complex and variable sugar profile is a hindrance.

Another highlight from **Paper I** was the mannitol content of *Halidrys siliquosa*, which could spark further studies on this species and confirmed the importance of data resolution. In terms of bulk composition, *Halidrys siliquosa* is not among the most interesting species, but the content of a single component (in this case mannitol) makes it potentially interesting. Therefore, more in-depth analyses are needed to either completely rule out species for biorefinery applications or to identify high-value components, as discussed further in section 6 in relation to **Papers II** and **IV**. At an early stage, bulk screening remains nevertheless essential as it directs the focus to those fractions, which could be interesting for various species, and estimates the amount of usable content. *Codium fragile* was originally part of the study, but it was not analysed for all components as it is an invasive species, with an ash content of 55%. Ash content is not a desirable trait for biorefinery, though there are some suggested uses of the minerals contained in seaweeds, as a salt or as an additive to improve combustion of other materials (Magnusson et al. 2016; Skoglund et al. 2017). An elevated ash content is especially limiting to biorefineries based on fractionation that rely on recovery of multiple product streams, as there is less overall organic material to recover.

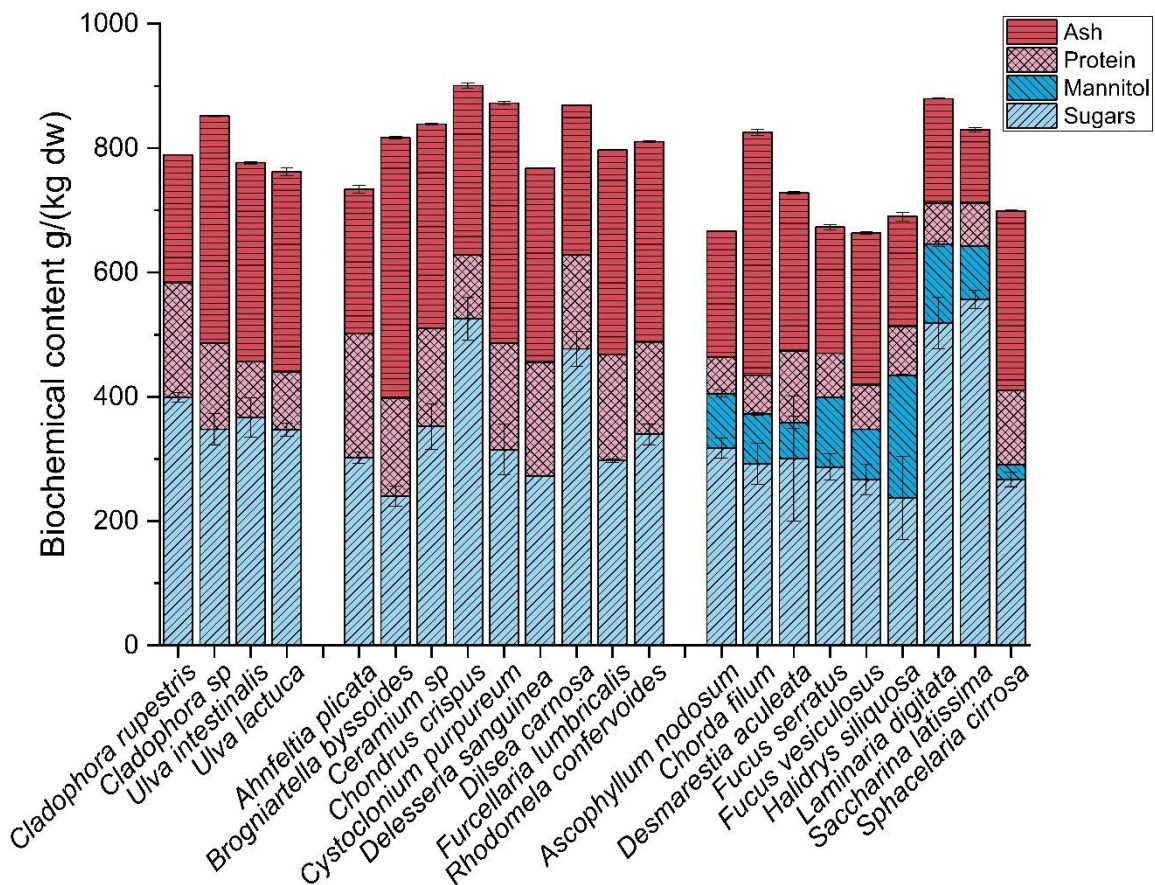


Figure 3. Composition of 22 seaweeds from the Swedish west coast belonging to the green (left), red (middle), and brown (right) macroalgae groups. Ash, protein, mannitol, and sugar content are shown based on the data presented in **Paper I**, Table 1.

Specific ash components

Even a low ash content could limit the potential of seaweeds, as it depends heavily on which constituents are found in this fraction. The levels of certain metals in several species in **Paper I** exceed the limits allowed for food and fertilisers in Sweden or the EU. The limit of 1 mg kg⁻¹ dw of cadmium for bio-fertilisers was exceeded by *Brogniartella byssoides*, *Cystoclonium purpureum*, and *Fucus serratus* and the Swedish limit for copper in eco-certified compost was exceeded by *Cladophora* sp. (Certifierad Återvinning 2018; KRAV ekonomisk förening 2017). The level of lead in vegetables is set at 0.1 mg kg⁻¹ w/w and was exceeded by all species except *U. intestinalis*, *C. crispus*, *C. purpureum*, *D. carnosa*, *A. nodosum*, *C. filum*, *F. serratus*, and *H. siliquosa* (European Commission 2018). The content of these regulated elements could possibly be dealt with by cultivation practices or selective sampling as metal content in seaweeds are known to vary by factors such as size, age and nutritional state (Hurd et al. 2014a). For biorefinery applications, these components need to be accounted for, but may represent a problem only if they accumulate in the end products. There are studies using seaweeds as a tool to clean contaminated waters from hazardous metals and HTL has been used to accumulate them in a solid, relatively inert form while producing bio-oil and recovering nutrients (Raikova et al. 2019).

4 Cultivation factors influencing composition and seaweed processing

To exploit cultivation conditions with the intent of maximising the valuable content and processing yields of desirable products from seaweeds it is necessary to know what factors influence seaweed composition and how. To this end, sufficient resolution is required to determine the effects on potential high-value compounds. This section describes the abiotic factors that are most relevant for cultivation in Sweden. Also, the findings from **Papers II** and **III** regarding how the abiotic factors impact composition and HTL processing of collected samples of *Ulva intestinalis* are summarised.

4.1 Important cultivation factors in Swedish waters and in tank cultivation

The number of factors impacting cultivation of seaweed is too large to fit in a single thesis. Hence, this work focused on those that are of particular relevance for cultivation of these species in Sweden, be it in tanks or at sea.

Salinity

Salinity is a highly variable abiotic factor in Swedish waters. As the Baltic Sea is isolated from the Atlantic by narrow straits between Denmark and Sweden, there is little input of oceanic water, whose salinity of about 35‰ is much higher than that of the Baltic Sea. As there is an output of low-salinity surface waters from the straits, the entire Swedish coastline becomes a long gradient of salinity ranging from around 2‰ in the north to 25–30‰ in the west (SMHI 2009b). This obviously has a large impact on marine life and the count of seaweed species count goes from 40 to around 250 along this gradient. The specific gradient for **Papers II** and **III** can be viewed in Figure 4.

Several studies have demonstrated the impact of salinity and salinity stress on the composition of seaweeds. An obvious effect of low salinity is increased turgor pressure due to the inflow of water caused by the lower solute concentration in the surrounding environment. This leads to a loss of ions and reduced organic solute concentrations.

Seaweeds utilise several different organic solutes, such as proline, sucrose, mannitol, and β -dimethyl sulfoniopropionate among others (Karsten 2012).

Ulva intestinalis, the focus species of **Papers II and III**, is well known for its resistance to varying levels of salinity. It inhabits a large vertical range along the shore and can grow in water with 0 to 102‰ salinity (Reed and Russell 1979), although the growth rate is reduced at both ends of the range. It can be found along the entire Swedish coast and is probably one of the most globally widespread seaweed species. A thicker cell wall has been reported in *Ulva intestinalis* sampled from rock pools (Wærn 1952), possibly reflecting an adaptation to dilute and/or concentrated sea water. At present, little is known about what influences cell wall components, and the observed variability is thought to be caused by a need for increased cell wall flexibility as the cell swells under low salinity conditions (Hurd et al. 2014c). Though there are few studies on salinity and its effect on biochemical composition, Floreto and Teshima (1998) studied the levels of soluble carbohydrates, protein and lipids at 10, 35 and 50 ‰. They saw the carbohydrate and protein content increase significantly while fatty acid levels decreased significantly at lower salinity.

Phosphorus and nitrogen

Two of the most important nutrients for seaweeds, which are often limiting in nature, are nitrogen and phosphorus. Figure 4 shows the summer average concentrations of nitrogen and phosphate near the sites sampled in **Papers II and III**. From these data, it is clear how both concentrations were much lower than the averages for seawater (30 μ M nitrogen and 2 μ M phosphate), which is to be expected during the summer period. What is really important for this thesis are the geographic differences indicated by these data. As indicated by the size of the error bars, nitrogen varies a lot over the course of summer. Anthropogenic input from agriculture and other human activities from the coastal regions surrounding Trelleborg (TBG), Åhus (ÅHS), and Karlskrona (KKR) has impacted the levels of phosphorus and nitrogen over the years (Rosenberg et al. 1990). This is reflected mostly by the levels of phosphorus in Figure 4. If the sampled *Ulva intestinalis* is nutrient-limited, differences in nutrient availability could mean that the limiting nutrient changes along the coast. Such a phenomenon has been reported previously along the Swedish coast (Rosenberg et al. 1990) and it could influence the composition of the samples in **Paper II**, just as nitrogen and phosphorus levels are shown to influence monosaccharide composition in **Paper IV**. McCauley et al. (2018) compared the lipid content in *Ulva* sp. grown under nitrogen starvation and saturated nutrient uptake concentrations. They saw increased fatty acid content under starvation conditions, but also a noteworthy increase in polyunsaturated fatty acids at saturated conditions. Phosphorus does not have the same overall effect, but seems to affect individual fatty acids (Floreto et al. 1996). Gao et al. (2018b) found that the protein and lipid content increased at elevated nutrient levels; whereas carbohydrate composition and content under varied nutrient levels have not been studied in detail.

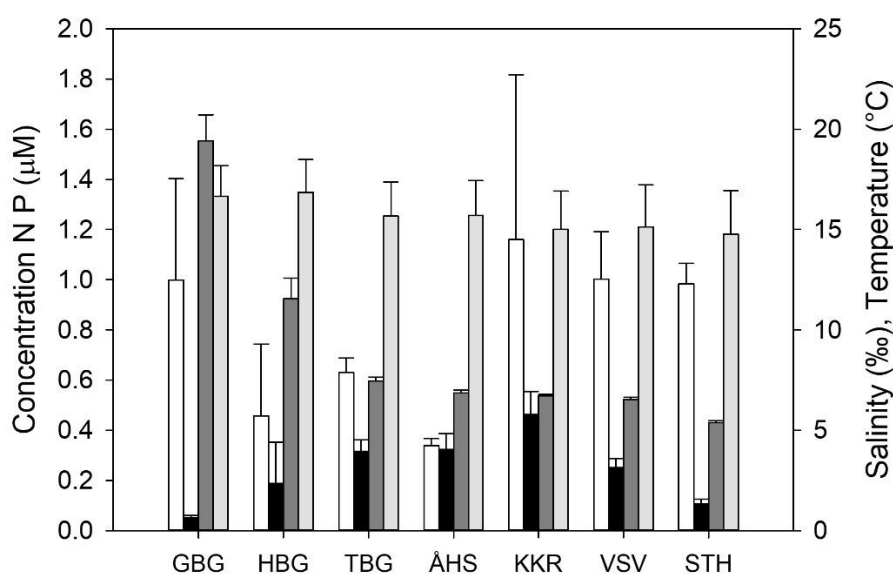


Figure 4. Summer averages (June-September) of nitrogen (white), phosphorous (black), salinity (grey), and temperature (light grey) in sea water (μM). Nitrogen is the sum of ammonium, nitrate, and nitrite concentrations (μM); whereas phosphorous corresponds to the phosphate concentration (μM). Data for nitrogen and phosphorus were extracted from environmental monitoring stations near the sampling points from Svenskt havsarkiv (Havs- och vattenmyndigheten 2019). Data for salinity and temperature came from the Nemo-Nordic ocean model (Hordoir et al. 2019). Means and standard deviations of the four months are shown (for TBG and VSV environmental data were available only for three months).

Temperature

As can be seen in Figure 4, temperature is one of the parameters that vary less in Swedish waters, or at least in the sampling area for **Papers II** and **III**. Previous reports described a positive effect on lipids and proteins as well as a lower ash content in *Ulva* spp. at elevated temperatures (20–25 °C) (Gao et al. 2018b; Liu and Zou 2015). While it is highly unlikely that the modelled 1–1.5 °C difference, using the Nemo-Nordic ocean model (Hordoir et al. 2019), could have any major impact on seaweed composition, it cannot be excluded *a priori*.

Irradiance

As Sweden is a long country stretching far in a north-south direction there will inevitably be differences in irradiance. The midnight sun in the north will, for example, provide 24 hours of sunlight per day, which is obviously more than in the south. However, in **Papers II** and **III**, only the southern part of Sweden was included, and the entire study area had approximately 1800 hours of sun a year (SMHI 2009a). Hence, the differences can be assumed to be minimal. In tank cultivation, however, this parameter can be manipulated by lights or shading, depending on what gives the biomass the wanted characteristic. For *Ulva* spp. it is previously known that irradiance increases lipid, lowers protein and influences carbohydrate content (Mhatre et al. 2019; Mohsen et al. 1973).

pCO₂

The concentration of dissolved CO₂ (pCO₂) has little relevance to cultivation in the sea as it cannot be controlled, but it represents an easy variable to change in tank cultivation. It is well known that the productivity of algae can be increased by increasing pCO₂ (Ip et al. 1982; Olischläger et al. 2013), and increases fatty acid and protein contents in *Ulva* spp. (Gao et al. 2018a).

4.2 *Ulva* spp. and identification issues

Over the course of this thesis, some issues related to the identification of *Ulva* spp. have arisen in the Baltic region. Species belonging to the genus *Ulva* have been subjects of discussion for many years regarding species identity and morphological features (De Silva and Burrows 1973; Wærn 1952). Recently, it was established that morphological features were not sufficient for identification, and sequencing of the plastid-encoded DNA barcoding marker *tufa* is required (Hughey et al. 2019; Steinhagen et al. 2019). This has revealed 9 species of *Ulva* along the German coast in the Baltic Sea, of which most could manifest a tubular morphology (Steinhagen et al. 2019). Because tubular morphology was generally associated with *Ulva intestinalis* along the Swedish coast, this new information prompted a detailed investigation of the species identity in the biomasses collected for **Papers II** and **III**. Indeed, the investigation revealed 4 samples to be *Ulva compressa* and the Tjö site containing 3 of the misclassified samples included in **Paper III** was omitted from **Paper II**. At the GBG site, 2 samples turned out to be *Ulva intestinalis* and they could be used in the study. The previous misclassification has to be kept in mind when discussing data from the published **Paper III**. Interestingly, the content of monosaccharides related to ulvan in the *Ulva compressa* samples was approximately half of that found in *Ulva intestinalis*. This indicates a large difference in total ulvan content between species and could motivate further investigation.

4.3 A study of *Ulva intestinalis* along the Swedish coast

Papers II and **III** used the same *Ulva intestinalis* material collected along the Swedish coast (see Figure 5), with one sampling point less as discussed in section 4.2. As seen in Figure 1 of **Paper III**, the missing point was located north of Gothenburg. The choice to study *Ulva intestinalis* was made partly from the compositional data obtained in **Paper I** and from published data regarding its high growth rate and wide distribution. The choice was also influenced by the two most interesting species from a compositional perspective, *Saccharina latissima* and *Laminaria digitata*, whose characterisation was already underway as part of the large collaborative Seafarm project.

The aim of the consortia behind **Papers II** and **III** was to first evaluate the composition of this species along the Swedish coast and gain insight into what might cause the observed variability. The relative amounts could then be used in HTL processing to derive a predictive model of bio-oil production. The sampling sites were selected to be as similar as possible with, *i.e.*, a hard bottom made of rock or stones, moving water, and no harbour of any kind in the immediate vicinity. Moreover, *Ulva intestinalis* was sampled at a depth of no more than a few decimetres. The exception to this was the HBG site, where *Ulva intestinalis* was sampled on rocks on a sandy beach due to the difficulty to satisfy all criteria along this stretch of coast. Because of this compromise, it was later realised that sand present in the tubular seaweed had not been cleaned properly and ended up contaminating the samples. Therefore, the statistical analysis performed in **Paper II** on monosaccharides and individual fatty acids was performed using data that had been converted to an ash-free dw. The sites were named based on the nearby cities of Gothenburg (GBG), Helsingborg (HBG), Trelleborg (TBG), Åhus (ÅHS), Västervik (VSV), and Stockholm (STH).

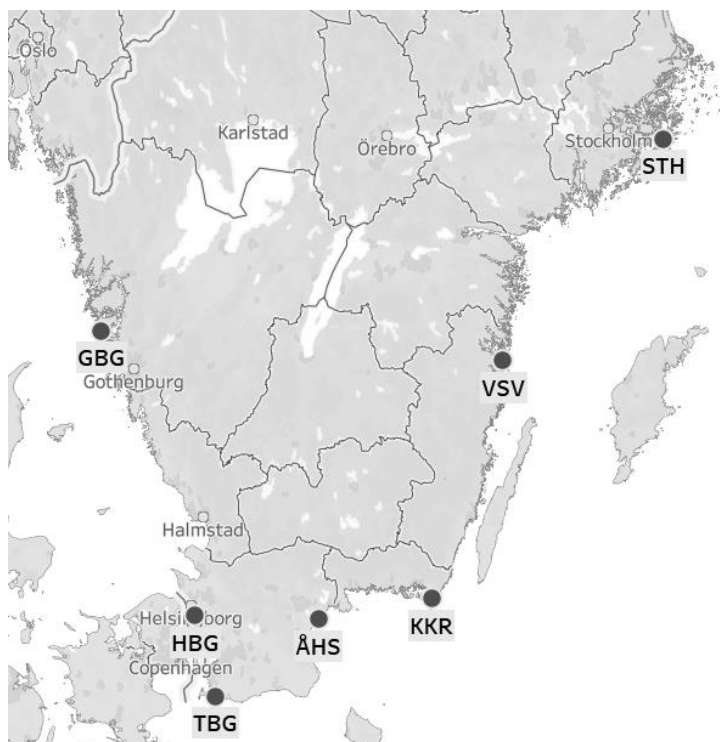


Figure 5. Map showing the sampling points of *Ulva intestinalis* included in **Paper II**.

Total composition of carbohydrates, fatty acids ash and protein

The overall composition can be viewed in Figure 6 and, as expected, ash and carbohydrates were the dominating components. All samples were in the same range as those of *Ulva intestinalis* shown in Figure 3 of **Paper I**. Given that the sample for **Paper I** was collected north of Gothenburg on the 1st of July while that for **Papers II** and **III** was collected in late August or early September, a possible influence of seasonality cannot be excluded. Overall, the values provide a clear indication that the reducing sugar assay was as good as HPAEC-PAD at measuring total carbohydrates. Statistical analysis revealed significant differences between some localities for all macrocomponents, or groups, except proteins (see Table 1 and 3 **Paper II**). Among carbohydrates, only the highest and lowest contents differed significantly between localities, with HBG (29% dw) and STH (41% dw), and all east coast localities at around 40% dw. This was in line with previous reports about carbohydrate content correlating negatively with water salinity (Floreto and Teshima 1998; Nielsen et al. 2016). Ash content was significantly higher in HBG (38% dw), compared to all other sites, whereas the lowest value was recorded along the east coast in STH (24% dw). Protein content tended to be, albeit not significantly, higher along the west coast ranging between 4.8–8.2% dw for all samples. Fatty acid content was significantly higher in GBG than at all other sites, with values ranging between 2.2–3.2% dw, which – unlike protein content – was in line with the results by Floreto and Teshima (1998).

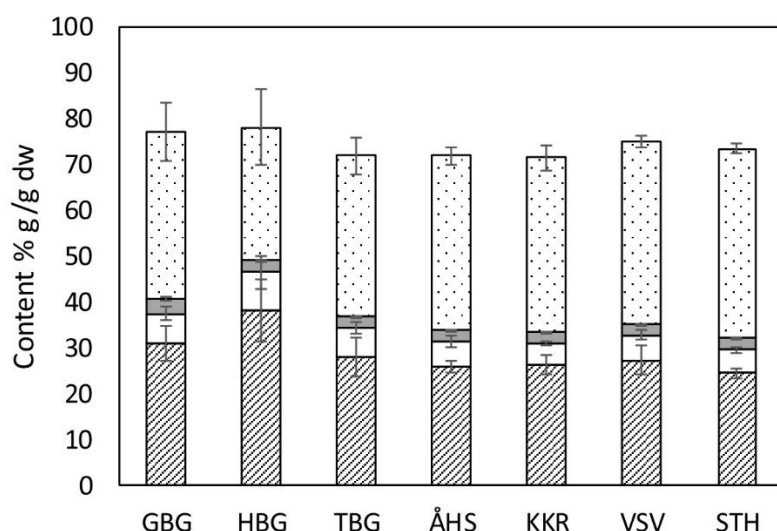


Figure 6. Biochemical components: ash (striped bars), proteins (open bars), total fatty acids (grey bars), and total carbohydrates (dotted bars) in *Ulva intestinalis* biomass from Tjörn (GBG), Helsingborg (HBG), Trelleborg (TBG), Åhus (ÅHS), Karlskrona (KKR), Västervik (VSV), and Stockholm (STH). Values are based on averages of three replicated samples (GBG duplicates) at each site with standard deviation as error bars.

Elemental changes

Carbon content of the biomass was lowest in HBG (22.2%) and highest in VSV and STH (29.4%) (see Figure 3 in **Paper III**), probably due to differences in ash content. Sulphur content was significantly lower in HBG than at all other sites and the average ranged around 4.7–7.9%. Generally, sulphur content increased as salinity decreased. Nitrogen levels were not statistically different between sites but were somewhat more elevated along the west coast. The SNK- tests (Table 3 in **Paper II**), revealed significant differences in Mg, Sr, Fe, S, and P content between locations. Phosphorus in particular exhibited an interesting pattern, with the following locations listed from lowest to highest: GBG (930 ppm), STH (1440 ppm), HBG (1540 ppm), VSV (1920 ppm), KKR (2300), ÅHS (2620 ppm), and TBG (2980 ppm). This pattern is close to the one seen for the environmental data in Figure 4. While not a surprise as *Ulva* spp. can accumulate polyphosphates (Hurd et al. 2014b), it showcases the ability of *Ulva intestinalis* to take up nutrients in eutrophicated environments.

Implications for processing

The purpose of the HTL process is to recover the energy in the treated material as a bio-oil that can then be upgraded. Therefore, yield and quality of the biocrude are of utmost importance to the process. In this work, yields ranged between 9 and 20% of the initial mass, with the lowest yield obtained from TBG and the highest from KKR samples, and the majority between 13 and 17%. No sites had statistically different yields, and variation within sites was sometimes greater than between them. The yields were low in comparison to those reported by other studies, which was likely due to a lower lipid and protein content as these components are preferentially recovered in the biocrude (Biller and Ross 2011; Raikova et al. 2017). However, no correlation was found in this study between biocrude yield and the content of carbohydrates, lipids, and proteins. The recorded yields corresponded to 29–55% of the total energy in the biomass based on comparisons of higher heating values calculated using the equation proposed by Channiwala and Parikh (2002).

The produced biocrude had several drawbacks and was in general of poor quality. The energy content ranged from 24.4 to 33.2 MJ kg⁻¹, which is considerably lower than that of a typical crude oil (42–44 MJ kg⁻¹). Also, both nitrogen (3–6%) and sulphur levels (0.9–4.4%) exceeded those found in crude oil (0.05–5% and 0.5–2.1%, respectively) (Ward et al. 2009), and correlated with biomass content. High nitrogen inhibits hydrodesulphurisation, which is required for high-sulphur oils, and causes NO_x emissions, while also requiring hydrotreatment prior to processing (Ward et al. 2009). A small proportion of total metals from biomass was found in the biocrude, but the value nevertheless exceeded that of crude oil considerably. Most problematic was the high level of iron in some biocrudes, which could cause plugging of catalyst beds and catalyst deactivation (Jarvis et al. 2016). See Table S1 in the supporting material of **Paper III** for a full breakdown of elements present in the biocrude.

Carbohydrates have been suggested to preferentially form aqueous phase products, but no such correlation was seen in **Paper III** (López Barreiro et al. 2015). The yields of aqueous phase products varied a lot within sites and ranged between 11.4 and 32.0%. The aqueous phase was found to be rich in Na, K, Ca, Mg, N, and P, and as such had been tested as a microalgae feedstock (Jena et al. 2011). Phosphorus can be recovered through precipitation from the aqueous phase, though with marginal economic benefit to the process (Papadokonstantakis et al. 2017). As with the biocrude, the levels of various elements varied highly within sites, but the trend was not completely in line with biomass content. For example, in VSV, K varied between 575 and 3872 ppm in the aqueous phase for the three samples, whereas variation in the original biomass was not as large (7200–9980 ppm). This kind of variation in composition of HTL products is unwanted, and a refinery would have issues with inconsistent products with these *Ulva intestinalis* samples.

Changes to high-value compounds

Proteins, ulvan or ulvan monosaccharides, and polyunsaturated fatty acids are the main potential high-value products from *Ulva* spp.. Changes in ulvan were indicated by alterations in the monosaccharides specific to it in *Ulva intestinalis*, i.e., rhamnose and iduronic acid. Rhamnose varied between 11.74 and 17.39% dw of the biomass content. Though not statistically significant, rhamnose was generally higher along the east coast. Iduronic acid ranged between 1.77 and 3.51% dw and followed the same trend as rhamnose. While in the case of iduronic acid, significance was at $\alpha = 0.1$, this was still not sufficient evidence of an increased ulvan content. Another indirect indication of a possible increased ulvan content came from analysis of sulphur content. The latter displayed the same trend as iduronic acid and ulvan is known for its sulphations, however the difference was again statistically significant only in HBG. Hence, results regarding ulvan remain inconclusive. However, as cell wall changes caused by salinity have been proposed before (Wærn 1952), the results obtained here motivate further investigation in a more controlled study.

The high-value fatty acid eicosapentaenoic acid (C20:5) has been associated with several health benefits. Here, it was found at 12–24 mg 100 g⁻¹ dw, with significantly lower concentration at the VSV and STH sites. The other total polyunsaturated fatty acids were not significantly different at the various sites, but the total levels (TPUFA) did differ. Specifically, their content was highest in GBG (580 mg 100 g⁻¹), which differed significantly from STH, AHS, VSV, and KKR, whereas the lowest value was recorded in KKR (310 mg 100 g⁻¹), which was significantly different from GBG and HBG. These results were in agreement with those of Floreto and Teshima (1998), who also observed less

polyunsaturated fatty acids at lower salinities, suggesting that salinity was the likely cause for their reduction.

From the available data in **Paper II** it cannot be determined where a potential cultivation site of *Ulva intestinalis* should be positioned. For this a larger analysis of a proposed biorefinery concept, probably based on fractionation, should be considered. Growth rate of the biomass and yields of the high-value compounds as well as their potential price would be essential to such a techno-economic analysis and requires further research. However, the scenarios of a lipid rich west coast biomass and a carbohydrate rich east coast biomass have emerged from the presented data.

East and west coast compositional differences

The data for the different components were analysed by principal component analysis (PCA) to visualise commonalities in the composition of monosaccharides, fatty acids, metals and the macrocomposition (Figure 7). Monosaccharides and fatty acids were analysed on an ash-free basis due to the previously mentioned contamination of sand in the HBG samples. As seen in the compositional variation of HTL products, within-site variation was evidenced also by PCA and no site formed clear clusters in all four plots. Monosaccharides confirmed the trends observed for rhamnose and iduronic acid, with east coast samples localizing to the right and in the middle, while some of the south points (HBG and TBG) grouped mostly to the left together with the two GBG samples. However, the division was not very clear and there were too few samples from the west coast to draw any overall conclusions.

The outlook was slightly better for fatty acids as both HBG and GBG grouped to the lower left quadrant of the PCA score plot, while the other sampling points were spread over the right and top side of the figure, with large variation within sites. Nevertheless, clear differences could be detected between the coasts, and the correlation plot indicates that total fatty acids levels are higher along the west coast. Unlike most polyunsaturated fatty acids, docosapentaenoic acid failed to follow a common trend and displayed an unclear pattern instead.

Metals and other elements probably showed the clearest division between east and west coasts, with GBG and HBG to the right and everything else spread from top to bottom on the left of the PCA score plot. One can discern the low sulphur levels in HBG in the bottom right corner, as well as an increase in the east coast samples and GBG somewhere in between. Magnesium exhibited substantial variation, which was reflected in the biocrude (see Table S1 in the supporting material of **Paper III**).

Lastly, macrocomposition PCA revealed a clear group in the top right of STH, VSV, and KKR. The samples in the south (AHS and TBG) were somewhat promiscuous and were split between groups. In contrast, west coast samples were spread all over the left side of the figure. This was caused by a large variation in fatty acid content between the two GBG samples and an exceptionally high nitrogen (protein) content in one of the three HBG samples. Based on this visualisation, a clear link between carbohydrate content and ash content emerged. Salinity most likely explained this link, as increased salinity results in higher ash content due to residual water, and low salinity has been shown to favour a larger carbohydrate content (Floreto and Teshima 1998).

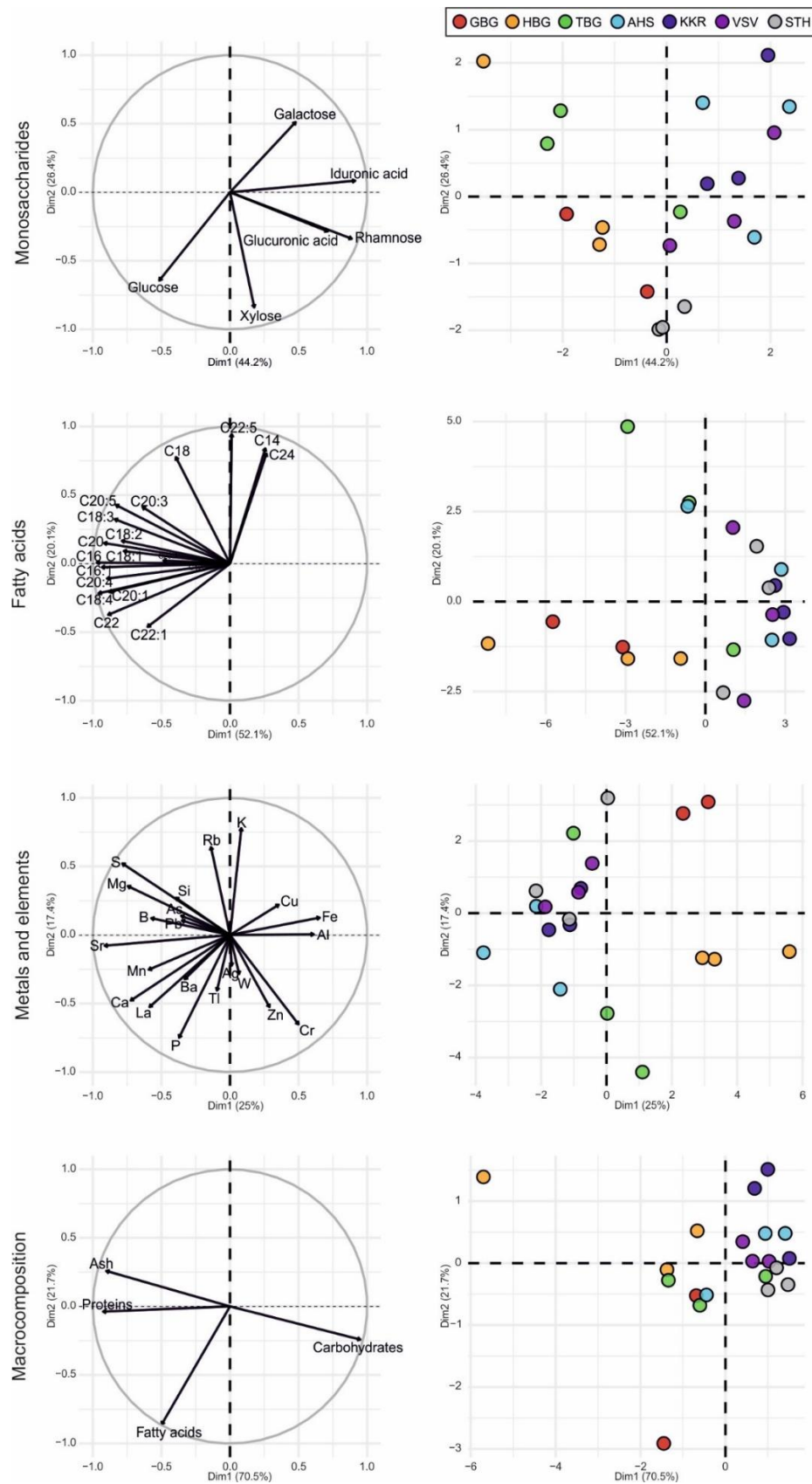


Figure 7. PCA analysis with correlation plots (left column) and score plots (right column) of monosaccharides, fatty acids, metals and elements and macrocomposition in *Ulva intestinalis* with locations listed by their position along the Swedish coast from Göteborg to Stockholm.

5. Maximising the valuable content of seaweeds

To control the composition of high-value compounds in *Ulva* spp., tank cultivation offers a suitable alternative as it allows precise control of cultivation conditions. If this can be used to fine-tune the biological activities of ulvan as well as its total content, tank cultivation could be highly beneficial to a biorefinery. Given the present lack of a market for ulvan and the limitations discussed in Section 2, it might be difficult for an ulvan-based biorefinery to turn a profit. One way to achieve this is by maximising the valuable content.

5.1 Effect of abiotic factors on the composition of *Ulva fenestrata*

Paper IV evaluated the impact of the abiotic factors irradiance (50, 100, and 160 $\mu\text{mol m}^{-2} \text{s}^{-1}$), temperature (13 and 18 °C), nitrate (3 levels), phosphate (2 levels), and pCO_2 (200, 400, and 2500 ppm) on *Ulva fenestrata*. Total carbohydrate content varied between 25.29 and 38.31% and was positively impacted by temperature and negatively by nitrate and pCO_2 , as seen by 2- and 3-level ANOVAs in Tables 1 and 2 of **Paper IV**. Among monosaccharides, changes to rhamnose and iduronic acid were the most interesting as these sugars are found exclusively in ulvan in *Ulva* spp. and they may represent potential end products. Rhamnose could for example be used to chemically produce rhamnolipids, which are bio-surfactants currently derived from fermentation by *Pseudomonas aeruginosa*. Iduronic acid could also be used for chemical synthesis (Mohamed and Ferro 2015) of glucosaminoglycans such as heparin. In fact, ulvan itself could potentially replace these products as it shares many biological activities with glucosaminoglycans. Rhamnose was impacted positively by irradiance and temperature, but negatively by phosphate and pCO_2 , and ranged between 4.62 and 6.92% dw. The combined effect of elevated temperature and irradiance could increase rhamnose content by 26% compared to when both factors were at their lowest levels. Iduronic acid was impacted positively by temperature, and negatively by nitrate and irradiance, with average content at 0.40–0.86% dw. Again, the combined effect of elevated temperature and irradiance augmented iduronic acid content by 70%.

The monosaccharide sequence and iduronic acid have been shown to be important for the biological activities of heparin and, given the latter's structural similarity to ulvan, they can therefore be assumed to be important for ulvan, too. Hence, the ratio of iduronic acid to glucuronic acid was investigated. The ratio was significantly affected by irradiance (higher at medium conditions) and nitrate (higher at ambient nitrate conditions). A higher ratio indicates more iduronic acid relative to glucuronic acid, which could be important when using *Ulva* spp. to extract iduronic acid as well as bioactive compounds. Specifically, the ratio was previously determined as 0.14–0.19 g g⁻¹ on purified ulvan from the same species collected from the local area as the strain used in this study (Wahlström et al. 2020). *Ulva* spp. biomass contains also some glucuronanpolysaccharides, although only in minor quantities (Lahaye and Robic 2007; Redouan et al. 2009).

The sulphation of ulvan is essential for its biological activities (Leiro et al. 2007). Therefore, the degree of sulphation was investigated by Fourier-transform infrared spectroscopy. As the resulting signal is not always constant between samples, the data for sulphate stretching at 845 cm^{-1} were normalised against P=O at 1240 cm^{-1} , which is typical of the fairly constant phosphodiester of nucleic acids and phospholipids (Mayers et al. 2013). The obtained values were then normalised again against the ulvan-specific monosaccharide rhamnose to provide an estimate of the degree of sulphation (see Tables 3 and 4 in **Paper IV**). This ratio was impacted by temperature, nitrate, phosphate, as well as the combined effect of nitrogen and phosphorus levels. The resulting data showed that the degree of sulphation increased significantly at low temperature and was significantly higher at ambient nitrogen and phosphorus compared to all other treatments affecting N and P experiments (Tukey's HSD, $p < 0.05$).

Alterations to ulvan structure could be potentially used to modify or fine-tune the biological activities of this promising high-value polysaccharide. More studies are required to confirm the observed changes and test how impactful they might be, before attempting to modify ulvan structure by altering cultivation conditions. Nevertheless, the large increases in potentially valuable monosaccharides represents an important finding and could be utilised to further optimise tank cultivation or select suitable cultivation sites at sea for *U. fenestrata*.

6. Methodological considerations

The choice of methods is much more ample than can be covered in this thesis. Therefore, this section focuses only on the methods employed in this work while skipping over other options. But, the complexity of seaweed composition, discussed in Section 3, and the difficult matrix with high concentration of salts makes seaweeds notoriously difficult to analyse. For these reasons, methods for measuring seaweed components are not as established as for lignocellulosic materials and part of the work in this thesis has been on method development.

6.1 Carbohydrate analysis of seaweeds

Measurement of total carbohydrates and monosaccharide profiles, which are the focus of this work, involves a number of steps regardless of methodology. In all cases, polysaccharides are usually hydrolysed into monosaccharides before being either measured in bulk or analysed in detail *via* high-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD) or high-performance liquid chromatography (HPLC). Several hydrolysis methods exist and a few variants were utilised in this work (Bikker et al. 2016; Manns et al. 2014) depending on the seaweed species being studied. Protocols reported in previous studies (van Wychen and Laurens 2013) were used to store hydrolysis samples. All these methods are based on a two-step scheme which starts with incubation of the material in 72% sulphuric acid, followed by dilution to 4% or 1 M sulphuric acid and further incubation at elevated temperature. These protocols are similar to classical hydrolysis protocols for lignocellulose and are good for the degradation of recalcitrant polysaccharides such as cellulose. However, such methods are also very harsh and can cause degradation of sensitive monosaccharides, such as uronic and iduronic acids. Methanolysis followed by hydrolysis using trifluoroacetic acid (TFA) has been shown to effectively hydrolyse ulvan while preserving uronic and iduronic acids (De Ruiter et al. 1992). It is much better than sulphuric acid at hydrolysing the glycosidic bond in certain ulvan dimers, but it is less effective at breaking up cellulose. To my knowledge no one has tried TFA hydrolysis on whole biomass. Nevertheless, the method could not be tried within this thesis work due to its inability to degrade cellulose. Once the hydrolysate is obtained there are several methods for measuring the resulting monosaccharide solution.

Reducing sugar assays for seaweeds

There are several different methods for measuring reducing sugars colourimetrically. Classical examples include the 3,5-dinitrosalicylic acid (DNS) assay, Nelson-Somogyi, and sulfuric-phenol methods, as well as the more recent 3-methyl-2-benzothiazolinone hydrazone (MBTH) method used in **Paper I**. In these methods the response comes from a reaction giving colour that can be used to calculate the concentration based on a standard curve made from a sugar of choice. The response factors for different monosaccharides can vary depending on the method. Algal carbohydrates are highly variable and often contain uronic acids as well as sugar alcohols. Sugar alcohols are not detected by these methods as they are non-reducing and must be measured by other means. Given that uronic acids can be measured only by a few methods, the choice of procedure is essential for seaweed hydrolysates, but their response factor can vary dramatically. For the sulphuric-phenol method, the response factor for uronic acids can be less than 50% of that of glucose (Herbert et al. 1971). In this context, the MBTH method is clearly superior as the response factor for uronic acids varies by only 20–25% compared to glucose (Anthon and Barrett 2002; Van Wychen et al. 2017). The variation in response for different hexoses is small with the MBTH method and the response for the pentose xylose differs by less than 10% from that of glucose. Therefore, when choosing a standard in **Paper I**, glucose was picked not only due to its response factor in the assay but also because it is very abundant in seaweeds (Table 2). Because, several species studied in **Paper I** had not been investigated at all previously, there was a risk of not identifying the monosaccharides correctly or of not having the right standards. Hence, the bulk method was chosen over HPLC or HPAEC-PAD for this study, which focused on screening many species.

HPLC

A standard method for measuring carbohydrates is HPLC and there are numerous variations regarding instruments, detectors, eluents, and protocols. The choice for this thesis was relatively straightforward as it fell on the setup available in the laboratory. This comprised a common HPLC system (UltiMate 3000, Thermo Scientific) coupled to a refractive index (RI) detector, a UV/VIS detector, and a Rezex ROA-Organic Acid H+ (8%) column (300 × 7.8 mm, Phenomenex). This system is run isocratically with 5 mM sulphuric acid and is mostly used for analysis of compounds in fermentation experiments. It is possible to analyse sugars on this system, but the main separation principle is ion-exclusion and as the pKa of sugars is usually around 12, they cannot be ionized at acidic conditions and will not bind to the ion-exchange resin. Fortunately, this system can be used to separate some sugars based on hydrophobic interactions and size-exclusion. This allows the easy separation of mannitol from other sugars, while many hexoses have overlapping elution times. Another problem with this system is the RI detector, which detects most analytes but at the expense of sensitivity. Given these issues, only mannitol was measured here by HPLC.

HPAEC-PAD

Anion-exchange chromatography allows for the separation of sugars with very sensitive detection. A high pH is applied to take advantage of most sugars being weak acids with pKas around 12. Therefore, sodium hydroxide is a common eluent for sugar analysis as it allows for at least a fraction of the sugars to be deprotonated. As the pKa is quite sensitive to the structure of the analyte, anion-exchange chromatography can be used to separate sugars with very small chemical differences, such as the epimers glucuronic and iduronic acids. The high pH also enables rapid oxidation of the sugar on the gold surface by pulsed amperometric detection, causing a cascading breakdown, from which a strong signal can be recorded. The laboratory is equipped with two HPAEC-PAD systems that can be used interchangeably

(Thermo Scientific Dionex, ICS-3000/ICS-5000). All work in this thesis was performed on a Dionex Carbopac PA1 4×250 mm column with a 4×50 mm guard. Method development and optimisation were carried out continuously on this system based on a standard protocol from the manufacturer, which could only separate common monosaccharides from lignocellulose. This method used sodium hydroxide and sodium acetate to prepare the column, whereas the actual separation was performed with water only. While good at separating hexoses and pentoses, this method could not detect and separate uronic acids, because their low pKa made them bind strongly to the column material and prevented their elution. As suggested by a colleague at the Royal Institute of Technology in Stockholm, a gradient of sodium acetate and sodium hydroxide was added during elution of the last hexose. After many trials, the method described in **Papers II** and **IV** was devised, allowing for the separation of all relevant sugars in a single run, as shown in Figure 8. While time consuming in comparison to a reducing sugars assay, the elevated resolution afforded by this strategy was essential to gain insight into the impact of growth conditions on potentially high-value sugars such as ulvan.

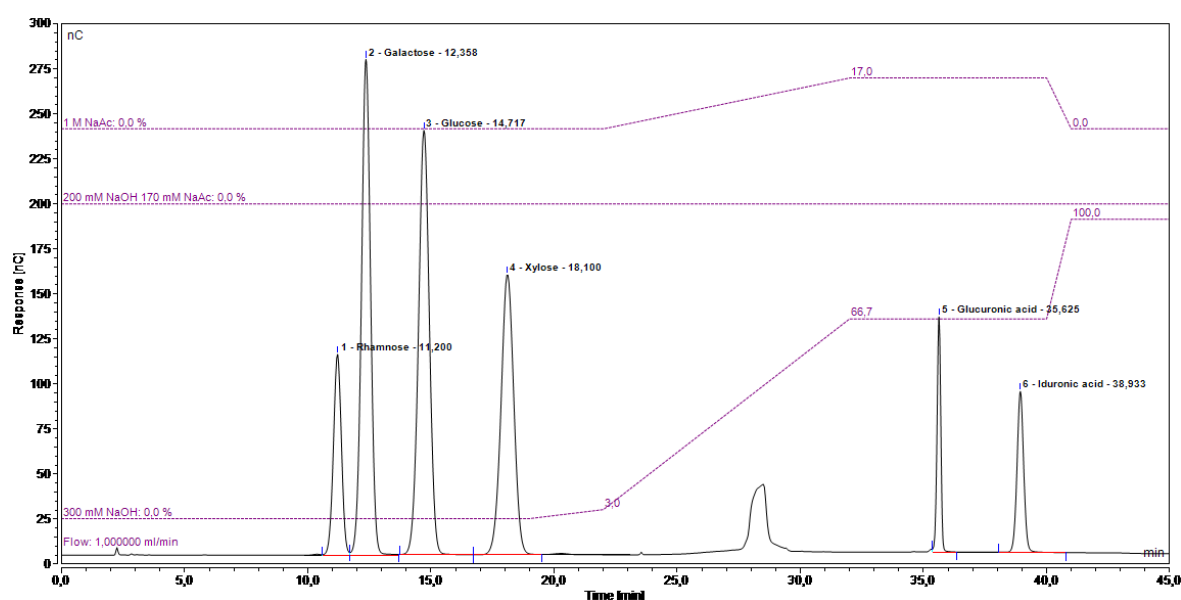


Figure 8. Standards analysed with the program used for analysis of *Ulva* spp. monosaccharides. In purple the gradients of three of the four eluents are shown and the remaining percentage is water. The peak at 28 minutes is of unknown origin, but likely caused by the gradient.

6.2 Protein analysis

Protein content is determined best by hydrolysis and single amino acids are best identified by chromatography techniques. However, protein solubilisation and detection by a general reagent (e.g., Lowry, Bradford) or determination of elemental nitrogen content followed by use of a nitrogen-to-protein conversion factor are quicker methods for screening purposes. Such conversion factors have already been determined for some seaweed species studied in this work. Nevertheless, nitrogen-to-protein conversion factors for seaweeds are troublesome as some species are known to accumulate nitrate (Young et al. 2007), and the amount of inorganic and organic nitrogen differs depending on growth location as well as season (Hurd et al. 2014b; Marinho and Holdt 2017). Therefore, the universal seaweed factor of 5 was chosen for all seaweeds in **Paper I**, as it corresponded to the average of many seasons, species, and localities according to Angell et al. (2016). In addition, using the same factor avoids the introduction of bias between novel and previously studied species,

facilitating comparison within the study. In **Paper II**, no such considerations were needed and the protein content was calculated with the factor 4.73, which had been measured on Norwegian *Ulva intestinalis* by Biancarosa et al. (2017).

6.3 Statistical analysis

Within this work, methods beyond regular standard deviation and analysis of variance have been employed to visualise as well as analyse the data. Some of the methods described below ended up not being utilised due to unforeseen circumstances as shortly discussed hereafter.

Principal component analysis (PCA)

PCA is a useful statistical tool for visualising complex data. It provides easier interpretation of trends or groupings within a dataset compared to just examining the raw data. By linearly transforming the data to a new set of axes (principal components, PCs) such that they account for as much variation in the data as possible, the dimensions can be reduced, allowing for a better overview of the data. Ideally, a few such components describe most of the variation. One commonly used method for making a rational choice regarding inclusion of PCs in the analysis is to make a scree plot, whereby the variance accumulated by the components is plotted against the number of components. If this plot does not show an elbow point, the data are likely not suitable for PCA, because absence of an elbow point provides no rational choice for how many components should be included in the analysis. On the contrary, if there is an elbow point, it gives the number of components to include in the analysis, as long as it accounts for enough variance. To be meaningful, the chosen number of components needs to account for about 70–90% of the overall variance (Jolliffe 2002). The final PCs can then be plotted by choosing any two components as axes, with the transformed data (scores) projected in the 2D diagram forming what is called the score plot. This plot is often combined with a loading plot into a final biplot. The loading plot is a vector plot, in which each variable has an eigenvector plotted for the chosen set of PCs and is calculated based on the maximum variance for that PC. In the loading plot, the vectors show the direction and weight of each variable for the PCs and, when combined with the score plot, it helps interpret which variables are causing separation or clustering of data points.

In **Paper I**, most data were unsuitable for PCA as there were either too few variables or the scree plot produced no elbow. PCA was found to be technically suitable for the analysis of metals; however, it did not reveal any interesting clusters. Though less insightful than it would have been had it revealed interesting commonalities, PCA nevertheless confirmed the diversity of seaweeds. PCA was also utilised in **Paper II** and discussed in depth at the end of section 4.3.

PERMANOVA

Permutational multivariate analysis of variance, commonly referred to as PERMANOVA, is a multivariate statistical test, mostly used in ecology, for testing statistical differences between groups. Unlike regular analysis of variance, PERMANOVA tests for similarity based on distance between data points instead of similarity between group averages. If the null-hypothesis assuming similarity is rejected, there is statistical difference between the groups. The p value is calculated from permutations of the data used to calculate the F statistic, which are then compared to the original F statistic. A permutation is a mathematical term for mixing and can easily be described by visualising a Rubik's cube, whereby the colour of the squares (the data) is always the same, but each turn of the puzzle produces a

different set of colours on each side, *i.e.*, a permutation. In this way, PERMANOVA tests if the random permutations are the same as the groups defined by the user. The method also allows *a posteriori* pair-wise comparison of levels of studied factors. For this work, PERMANOVA analysis was planned to be a part of **Paper II**, but data for one whole site and a single sample from another had to be discarded as they were eventually found to be a different species. This left too few data points for the west coast to achieve any meaningful statistical insight from comparing it to the east coast data by PERMANOVA.

Canonical analysis of principle coordinates (CAP)

Similar to PCA, CAP offers a way of visually representing statistical data. The fundamental difference between the methods is that CAP is constrained by the data being placed in pre-defined groups and the analysis aims to find the axes through the cloud of data that are best at discriminating these groups. The method goes well with PERMANOVA, which can confirm significant differences between the groups to motivate using this constrained method. The rationale for using CAP over unconstrained methods such as PCA is that when a PCA maximises the variation described by the individual axes, to reveal existing group differences, it can fail to do so in situations where the variation within groups is larger than that between them. The risk with CAP is that it may lead to overparametrisation, resulting in overfitting of the data and spurious relationships. To overcome this issue, diagnostic tools such as PERMANOVA are applied. Similarly to PCA, a set of axes that accounts for around 60% or more of the variation is produced. To validate if an appropriate number of axes has been chosen a misclassification procedure is utilised. In this diagnostic, data points are removed from the analysis, which is then rerun, followed by them being allocated to the group whose average is closest in the canonical space. When repeated for all the points, a misclassification error is calculated based on the percentage of data points not classified in its own group.

As mentioned previously for PERMANOVA, CAP was supposed to be used for data analysis in **Paper II** but due to the lack of data points the misclassification error was too large for this analysis. Additionally, the plots produced added no more separation of groups than PCA plots. Therefore, this analysis is not included in this thesis.

7. Conclusions

Seaweeds could become an important piece in the puzzle of a future bioeconomy. Within my work, there are several small discoveries that could help pave the way for biorefineries based on seaweeds and, especially, the bioactive polysaccharide ulvan.

There are plenty of potential seaweeds that could be utilised for biorefinery, but in **Paper I** the broad screening of bulk compounds found few surprises among the species with the most beneficial composition for biorefining. Although the evaluation was limited by the bulk assessment of compounds, the most interesting species were *Saccharina latissima* and *Laminaria digitata*. These species are being investigated in numerous research facilities across northern Europe for their potential applicability in biorefinery and companies have started utilising these seaweeds for food. *Chondrus crispus* is already used in the production of carrageenan and this could be a potential application for *Dilsea carnosa* as well. The most novel finding was the notable mannitol content in *Halidrys siliquosa*, which could motivate further investigation into this largely overlooked species. This finding in **Paper I** also shows the importance of detailed data resolution for the evaluation of seaweeds, as *Halidrys siliquosa* would likely have been overlooked if not for the HPLC analysis of brown seaweeds to determine their mannitol content.

Papers II and **III** produced some novel information about *Ulva intestinalis*. This species was found to be unsuitable for production of crude bio-oil by HTL. The resulting oil was of low quality due to a high sulphur and nitrogen level as well as insufficient energy content compared to commercial oils. Most likely, HTL processing of *Ulva* biomass is best suited for residuals from a biorefinery or biomass of low quality such as beach cast seaweeds.

Interesting variations in monosaccharide content were observed along the Swedish coast. While guessed at already in the 1950s and 60s through ocular inspection, **Paper II** provides further indications of changes to the cell wall of *Ulva intestinalis* along a salinity gradient. Though only iduronic acid shows significance at $\alpha = 0.1$, the trend of elevated rhamnose, iduronic acid and sulphur all point at an elevated ulvan content in Baltic Sea. The increase in ulvan content could be of great economic importance to a biorefinery centred around this component. Therefore, the salinity's effect on the ulvan content and structure should be further explored under more controlled circumstances, which would also confirm if the

indications in **Paper II** are true. Contrastingly, the valuable polyunsaturated fatty acids had significantly higher levels on the west coast.

In **Paper IV**, *Ulva fenestrata* was grown under varying abiotic conditions in tanks. We could see indications of changes to the degree of sulphation, as well as the amounts of specific monosaccharides present in the ulvan fraction. Iduronic acid, which is important for the biological activity of heparin, became more abundant at intermediate irradiance and elevated temperature (70% increase). Hence, it is safe to assume that changes in cultivation conditions most likely altered the bioactivity of ulvan as the varying fractions are important for the bioactivity of sulphated polysaccharides.

8. Future perspectives

The work in this thesis has applied the very practical mindset of an engineer to a multidisciplinary project spanning ecology and bioscience. This practical and result-driven focus is most notable in **Paper II**, which does not fully explore the mechanistic reasons for the observed differences, but rather uses the findings to shed light on the promising potential of seaweed cultivation in Sweden and points to several questions that could be addressed in future studies.

The screening study (**Paper I**) offers ample reason to investigate more species and to do so with greater detail. This could require the development of additional tools for the evaluation of seaweeds that offer a rapid yet deeper insight than bulk analysis. One such method could be Fourier-transform infrared spectroscopy, which has already been used to some extent for seaweed screening but requires further development. This paper also shows that additional analysis should be performed on some of the species. *Dilsea carnosa*, for example, showed similar sugar contents to commercially utilised *Chondrus crispus* and, depending on the type of carrageenan, it could be tested in cultivations for the production of carrageenan biomass in Europe.

Paper II leaves open the issue of whether salinity is causing the compositional changes seen in the study. Additionally, it remains to be determined if the actual structure of the carbohydrates are impacted by the variable environmental conditions. The Baltic Sea offers an interesting location for seaweed cultivation, not only to produce valuable biomass, but also for bioremediation through the removal of phosphorus and nitrogen. Sea-based cultivation of *Ulva* spp. is being investigated and should be tested in the Baltic Sea to evaluate the specific challenges posed by this environment, such as the potential colonisation of other species on the cultivars or the availability of substrate. The growth rate in the Baltic Sea could turn out to be significantly lower compared to that on the west coast; however, considerations about the growth rate are just the start when choosing whether to cultivate *Ulva intestinalis* in Swedish waters. The market for the potential products has to be present before there is a reason to cultivate *Ulva*. This can be a long and costly process within the medical field, where several of the potential applications lie. However, there are unexplored concepts regarding synthesis of valuable compounds from iduronic acid and rhamnose that could be worth exploring. A full concept needs to be evaluated in a techno-economic analysis to be able to conclude what conditions are best for cultivation. Such a study would benefit

from the inclusion of HTL data. Though not directly suitable for *Ulva intestinalis* biomass, HTL could be relevant for the creation of additional products from a biorefinery from side streams.

Paper IV identified changes to the amount and structure of ulvan. These must be further confirmed and their impact on the biological activity of polysaccharides should be assessed. Changes in iduronic acid content and sulphation differences likely affect antioxidant and antithrombotic activity. Ulvan structural changes could potentially be linked to effects on bioactivity, thereby allowing for fine tuning of ulvan towards specific properties already at the cultivation stage. The type of control required in this case would likely make tank cultures on land more suitable than sea-based cultivation but would require scaling-up the production. Various types of closed bio reactors, with a capacity of thousands of cubic metres, exist for microalgae, enabling full control of all the parameters impacting cultivation. For highly specialised medical applications this type of production probably needs to be developed for *Ulva* spp. to enable standardisation of biomass quality and content. This key issue identified by Hafting et al. (2015) promotes further studies of abiotic factors impact on composition, especially in novel production systems.

Several biorefinery concepts have been suggested for *Ulva* spp. and ulvan extraction, but they have been seen to negatively impact the bioactivity of the isolated ulvan (Kidgell et al. 2019) and remain at a very small scale. A recent work by Konasani et al. (2018) identified a novel ulvan lyase and enzymes acting on ulvan are emerging as an active area of research. Such enzymes could improve the extraction of ulvan as a whole or aid in the preparation of bioactive oligosaccharides.

I firmly believe that the seaweed industry is going to expand over the next few decades. The low hanging fruit to expand the industry is to produce seaweeds for food and, as that is a high-value application with minimal production costs, it is up for the industry to choose a starting point. However, there are many areas in which seaweeds could make a significant contribution in replacing less sustainable products. In the projects I have been part of, interesting materials have been brought forward. These materials made from seaweed polysaccharides warrant further research as possible alternatives to plastics. To exploit the full potential of seaweeds, I have suggested some ways of continuing my work (see above); however, a large breakthrough will occur only through technical development of novel applications, which can turn seaweed cultivation into a large-scale global industry. For now, seaweed biomass production is too expensive in developed countries and products from feasible biorefineries too cheap. If the biomass price were reduced through technical innovation, a potential business case could be made for the use of marine algae for something other than food.

Another problem with seaweeds is tying the value chain together for sea-based cultivations. Any cultivation not done in the tropics will face issues of seasonality. *Saccharina latissima* for example is usually put into the sea during the autumn and harvested in late spring or early summer depending on site conditions. Unlike land-based crops, where methods and infrastructure for drying and storage are already present, the seaweed industry will have to invent efficient techniques for the storage of biomass. While drying is easily done for small amounts of biomass, preserving biomass for a biorefinery that might need 10 000 tons per day is a different matter. Here, large scale methods that preserves the compounds of interest are needed.

Personally, I think the economic case for seaweed cultivation and refining has to improve further before the industry can take off on a large scale. Mostly development is needed in the product department and this is a hot research topic already. Nevertheless, at some point, the economic case may become strong enough for a pioneer to take a chance and start a novel seaweed industry.

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To do a PhD was nothing I ever planned, but a master's thesis turned into a project assistant role that later turned into a PhD and it has been a crazy ride. Looking back, it has been some kind of emotional rollercoaster and I've been glad, sad, happy, angry, surprised, triumphant, terrified and grief struck. This ride is not one I would ride again, but I am very happy to have ridden it. I have a whole lot of people to thank for their support and friendship that has gotten me to this point.

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